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MOLECULAR APPROACHES TO UNDERSTANDING NEURAL NETWORK PLASTICITY AND MEMORY: THE KAVLI PRIZE INAUGURAL SYMPOSIUM ON NEUROSCIENCE

M. SANDER,[a] L. H. BERGERSEN[b] AND J. STORM-MATHISEN[b]*

[a]*Page One Editorial Services, 685 Poplar Avenue, Boulder CO 80304, USA*

[b]*Department of Anatomy, Institute of Basic Medical Sciences, and Centre for Molecular Biology and Neuroscience, University of Oslo, PO Box 1105 Blindern, 0317 Oslo, Norway*

Abstract—The Kavli Prizes were awarded for the first time in Oslo, Norway on September 9, 2008 to seven of the world's most prominent scientists in astrophysics, nanoscience and neuroscience. The astrophysics prize was awarded jointly to Maarten Schmidt, of the California Institute of Technology, USA, and Donald Lynden-Bell, of Cambridge University, UK; the nanoscience prize was awarded jointly to Louis E. Brus, of Columbia University, USA, and Sumio Iijima, of Meijo University, Japan; and the neuroscience prize was awarded jointly to Pasko Rakic, of the Yale University School of Medicine, USA, Thomas Jessell, of Columbia University, USA, and Sten Grillner, of the Karolinska Institute, Sweden. The Kavli Prize is a joint venture of the Kavli Foundation, the Norwegian Academy of Science and Letters, and the Norwegian Ministry of Education and Research.

The Kavli Prize Inaugural Symposium on Neuroscience was held at the University of Oslo on 8 September, 2008, organized by L.H. Bergersen, E. Moser M.-B. Moser, and J. Storm-Mathisen. At this Symposium, seven leading neuroscientists described their groundbreaking work, which encompasses some of the most important recent advances in the field of neuroscience, from molecule to synapse to network to behavior. The Symposium was a fitting tribute to Fred Kavli's vision of neuroscience as an outstanding area of progress, and to the achievements of the winners of the first Kavli Prize in Neuroscience. The main points of the Symposium presentations are summarized below. © 2009 IBRO. Published by Elsevier Ltd. Open access under CC BY-NC-ND license.

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The field of neuroscience has experienced explosive progress in the last decade, fueled by important and unexpected discoveries in the areas of protein structure and function, neural plasticity, neural networks, adult neurogenesis, learning and memory (Table 1). Some of these recent discoveries went strongly against the grain of established dogma in the field of neurobiology. For example, early models of brain function proposed that adult brains were relatively immutable, and that neural networks in the adult brain could be simply described as a static set of connections, akin to a wiring diagram in an electrical device. In contrast, speakers at this Symposium repeatedly emphasized the concept of neural plasticity, which is critical not only during normal brain development, but also for essential functions of the adult brain, including learning and memory. The hippocampus (Fig. 1A) is the focus of much research on learning and memory, because animals with experimentally-induced total or region-specific hippocampal dysfunction demonstrate gross or selective loss of capacity to process information and learn (Fig. 1B). Environmental stimuli modulate brain structure and function, such that an enriched environment and physical activity increase the rate at which new brain cells and new synaptic connections form and persist in the adult brain. Many ongoing studies use rodent model systems, and these systems allow neuroscientists to apply powerful molecular tools to analyze brain structure and function. Sophisticated use of transgenic mouse strains and engineered viruses, as well as advances in imaging techniques, allow researchers to analyze the properties of individual neural cells, to measure activity at individual

*Corresponding author. Tel: +47 97193044; fax: +47-22851278.
E-mail address: jon.storm-mathisen@medisin.uio.no (J. Storm-Mathisen).

Abbreviations: AB, anterior burster neuron; GFP, green fluorescent protein; KO, knockout; LTP, long term potentiation; LP, lateral pyloric neuron; MHC1, major histocompatibility complex class I; PD, pyloric dilator neuron; PIRB, paired immunoglobulin-like receptor B; PY, pyloric neuron; SPM, synaptic plasticity and memory; STG, stomatogaster ganglion; β2M, β2-microglobulin.

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Table 1. Symposium highlights

Neurotransmitter transporters couple energetically-disfavorable transport of one substrate to energetically-favorable co-transport of a second substrate or ion. Crystallography reveals how atoms in the protein interact with substrate and ions to effectuate transport.
Ocular dominance plasticity, which is subject to positive and negative regulatory control during development, is downregulated by an MHC1/PIR-B-dependent pathway. Thus proteins governing the immune system also contribute to the tuning of brain function.
Neural circuit parameters in biological systems display a significant amount of cell-to-cell and animal-to animal variation, without significant degradation in circuit performance.
Adult neural stem cells, which reside in the subgranular zone of the hippocampal dentate gyrus and the subventricular zone of lateral ventricles, are pluripotent cells that are capable of self-renewal. Adult hippocampal neural stem cells give rise to neurons and astrocytes in a context-dependent manner, and form functional synapses that preferentially integrate with pre-existing circuits in the dentate gyrus.
The synaptic plasticity and memory hypothesis suggest that LTP is a physiological correlate of memory; this hypothesis is being tested by manipulating synaptic responses in memory-specific neural subnetworks.
Glutamatergic synapses play a key role in hippocampus-dependent learning. Specific AMPA and NMDA glutamate receptor subunits and subtypes in the hippocampus play differential roles in spatial working and spatial reference memory.
The trisynaptic pathway in the hippocampus is required for rapid one-time contextual learning, but is dispensable for slow multi-trial spatial tuning and other associative memory tasks in mice with a functional monosynaptic pathway in the hippocampus.

synapses, to manipulate gene expression in specific cell types or specific brain subregions, and to image brain structures with breathtaking resolution. For example, long term potentiation (LTP) can now be measured at a single synapse, individual adult-born neurons can be visualized at multiple time points after their emergence in the dentate gyrus, and glutamatergic receptors, or even specific glutamatergic synapses, can be selectively inactivated in the entire forebrain or in subfields of the hippocampus.

The "ultimate" understanding of function at the molecular level is how the individual atoms of proteins determine the proteins' actions, exemplified by Eric Gouaux's work on the structure of glutamate transporters, which are essential for the performance of glutamatergic synapses. This level of insight is a necessary basis for understanding brain function and pathology as well as for rational drug design. Surprisingly, and somewhat analogous to the multipurpose roles of glutamate, the major histocompatibility complex (MHC) molecules of the immune system also modulate brain function, providing a mechanism that limits activity-dependent neuronal plasticity, as shown by the work of Carla Shatz. The buzzword most commonly mentioned during the Kavli Prize Neuroscience lectures was "activity-dependent plasticity", including the formation of new neurons in the adult brain, championed by Fred Gage. One of the most striking new developments in neuroscience research is the successful application of molecular techniques at the level of the single cell. The dogma of hard-wired neural circuits is a thing of the past. It is also noteworthy, as shown by Eve Marder, that networks with widely different single unit properties can provide similar network output and behavior, indicating that multiple routes may lead to successful adaptation. As emphasized by Timothy Bliss at this Symposium, and recapitulated by the work of Peter Seeburg and Susumu Tonegawa, a challenge of the future is to understand how groups of neural cells work together as neural subnetworks, enabling critical brain functions such as memory and learning. A beginning to this is the dissection of how different glutamate receptor types (Seeburg) and different hippocampal synapses subserve different memory functions. Thus the multisynaptic entorhino-hippocampal circuit through the dentate gyrus and CA1 is required for one-trial learning of a new situation, as opposed to slow multi-trial learning (Tonegawa). Could the adult neurogenesis in the dentate gyrus (Gage) be somehow involved in this clearly important function? In the realm of neural networks, an exclusively reductionist approach may not be sufficient to reveal how a normal brain functions and the causes of brain dysfunction, when it occurs. Undoubtedly, future molecular analyses at the level of the single cell or synapse, and at the network level, will continue to remodel our concept of human brain structure and function.

SYMPOSIUM SEMINAR PRESENTATIONS

Eric Gouaux Portland, OR, USA

Structure and mechanism of neurotransmitter transporters. Communication in the CNS of mammals is effected by a gradient in the concentration of neurotransmitters, such as acetylcholine or glutamate, in the intercellular space between pre-synaptic and post-synaptic neurons, also called a neural synapse. Rapid and faithful synapse function requires cycles of neurotransmitter release, as well as a mechanism to clear neurotransmitter from the synaptic cleft. The latter function is provided by neurotransmitter transporters.

Neurotransmitter transporters are found on neural glial cells, as well as on pre-synaptic and post-synaptic neurons. Because most neurotransmitters are polar molecules, and because their concentration is usually higher inside than outside the cell, transport of a neurotransmitter across the membrane and against a concentration gradient occurs despite energetic and thermodynamic barriers. Peter Mitchell, in a seminal paper on membrane transport theory, proposed that these barriers might be overcome by coupling energetically-disfavorable transport of one transporter substrate to energetically-favorable co-transport of a second substrate or ion. This model requires that binding of one substrate influences binding of the second substrate. Recent studies have extensively validated this model and provided evidence that neurotransmitters are transported against a concentration gradient by coupling their transport with transport of one or more ions, most commonly sodium ions, whose intracellular concentration is typically very low.

The above model predicted that neurotransmitter transporters would minimally adopt two conformations: an "open-to-the-inside" conformation and an "open-to-the-outside" conformation. However, the X-ray co-crystal structures of LeuT and Glt_{Ph}, bacterial orthologs of eukaryotic neurotransmitter transporters for glycine/GABA/dopamine/

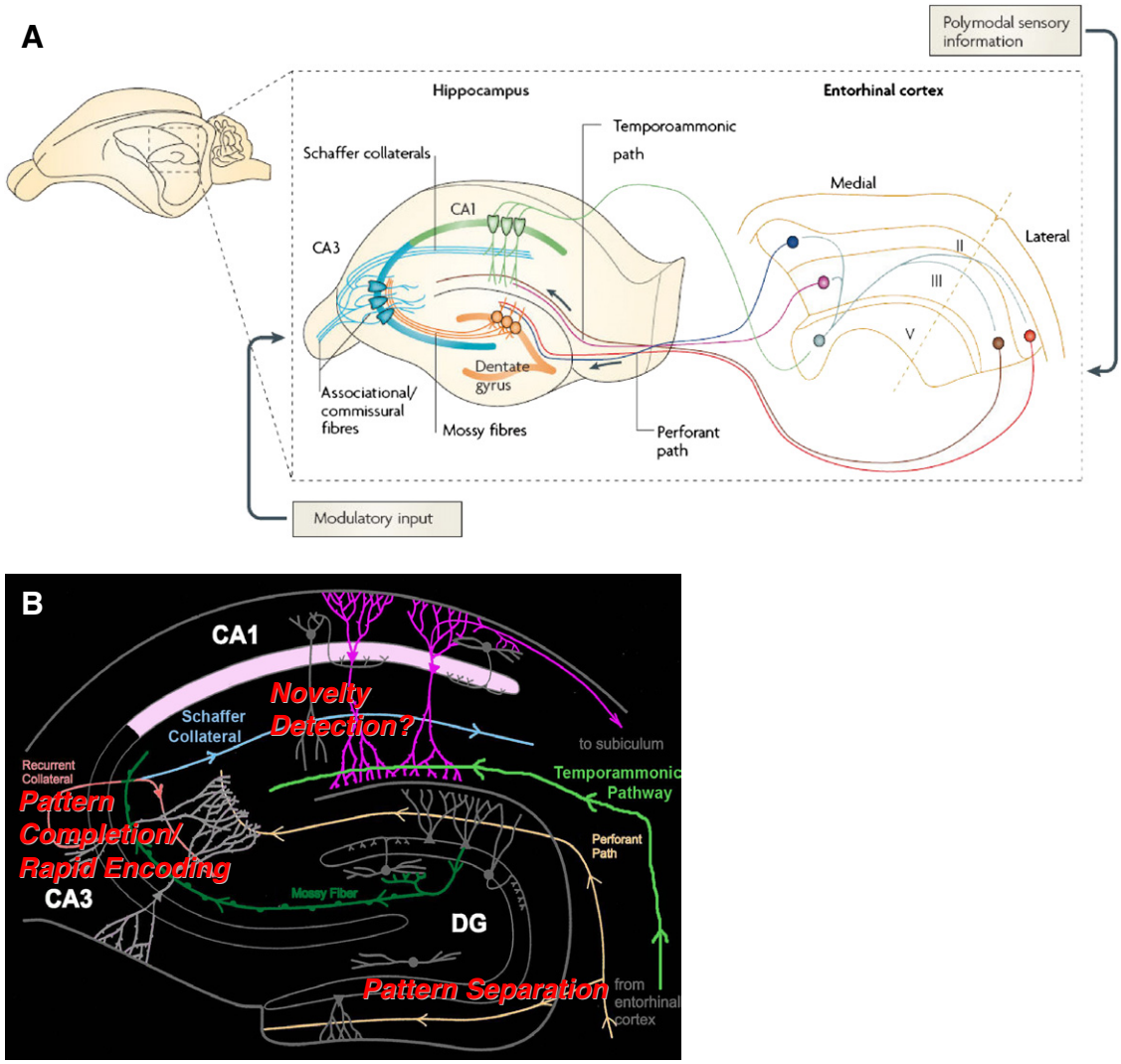

Fig. 1. A. The hippocampus. The primary excitatory circuit in the hippocampus is a trisynaptic loop, in which axons of the perforant path convey information from neurons in layer II of the entorhinal cortex to dendrites of granule cells in the dentate gyrus. Axons of the dentate gyrus granule cells (i.e., mossy fibers) project to proximal apical dendrites of CA3 pyramidal cells which, in turn, project to ipsilateral CA1 pyramidal cells through Schaffer collaterals and to contralateral CA3 and CA1 pyramidal cells through commissural connections. CA1 pyramidal cells are also innervated by layer III cells of the entorhinal cortex, forming a monosynaptic loop back to layer V of the entorhinal cortex. The three major subfields of the hippocampus, CA1, CA3 and dentate gyrus, are tightly packed in an interlocking C-shaped arrangement. Multiple inhibitory neurons (not shown) also exist in the hippocampus. (Adapted with permission from Neves et al., 2008 *Nat Rev Neurosci*.) B. Subfield-specific functions of the hippocampus in learning and memory. Using hippocampus subregion specific NMDAR knockout mice, Susumu Tonegawa's laboratory demonstrated subfield-specific functions in learning and memory. The dentate gyrus plays a dominant role in pattern separation; CA3 plays a dominant role in rapid encoding and pattern completion; and CA1 plays a dominant role in novelty detection. (Provided by Susumu Tonegawa.)

5-HT/norepinephrine and glutamate/aspartate/alanine/serine/cystine, respectively, showed that the two-state transporter concept was incorrect. Structural studies of co-crystal complexes of Glt_{Ph}, an archaebacterial Na^+1-coupled glutamate/aspartate transporter from *Pyrococcus horikoshii*, unexpectedly revealed that aspartate and two sodium ions are completely buried within a polar chamber located halfway across the membrane bilayer formed by the tips of helical hairpins HP1 and HP2. This polar pocket in Glt_{Ph} is not accessible to the extracellular or intracellular space and is "gated" via movement of HP2. Two sodium ions, localized near the bound aspartate, are coordinated primarily by carbonyl oxygens in TM7, TM8, and HP2. Aspartate binding isotherms confirmed that binding of sodium and aspartate to Glt_{Ph} is tightly coupled, such that a 15-fold increase in sodium concentration correlates with an approximately 1000-fold increase in affinity for aspartate. Most remarkably, similar studies of bacterial LeuT revealed a buried substrate binding site, local structural similarity to Glt_{Ph} in the vicinity of bound sodium ions, and highly co-operative ion-substrate binding behavior, despite lack of protein sequence homology between LeuT and Glt_{Ph}.

Thus, the structures of Glt_{Ph} and LeuT, and a large body of additional data suggest that secondary transporters exist in three states: open to the outside, occluded, and

open to the inside. Furthermore, the data suggest a transport cycle, in which (1) substrate and ion bind cooperatively to the transporter in the open-to-the-outside state; (2) the transporter undergoes sequential conformational transitions to the occluded (occupied) and open-to-the-inside states; (3) substrate and ion dissociate from the transporter; and (4) the transporter cycles to an occluded (empty) state.

Extensive binding surveys showed that all transportable ligands bind to LeuT in the occluded (occupied) conformation, while non-transportable ligands (i.e., competitive inhibitors), such as tryptophan, stabilize LeuT in the open-to-the-outside conformation. Because many members of the superfamily of secondary transporters (Fig. 2), including antiporters such as ApcT (a bacterial homologue of the glutamate/cystine antiporter), share a high degree of structural similarity with and adopt a nearly identical protein fold as LeuT, it has been proposed that molecules that inhibit transport may share a common mechanism, in that they bind to and stabilize the open-to-the-outside conformation of the transporter. This hypothesis has significant therapeutic implications, because it could facilitate rational design of inhibitors for a wide range of proteins, including transporters at excitatory and inhibitory neural synapses.

Carla J. Shatz Stanford, CA, USA

Moonlighting MHC1 and brain circuit tuning. Human experience, such as sensory input, can induce changes in neural circuits, a phenomenon known as activity-dependent neural plasticity. This process and its physiological/molecular correlates have been extensively analyzed in the context of ocular dominance, a property of the visual system of primates and other mammals, including mice. As the visual system develops, left- and right-eye specific neurons form segregated connections to the lateral geniculate nuclei (LGN), and eye-specific LGN axons, in turn, form connections with eye-specific columns in layer 4 of the primary visual cortex. Equal spaced eye-specific columns form when input from the left and right eyes is equal during critical developmental stages of the visual system. In contrast, left- or right-eye visual deprivation during the critical periods leads to asymmetric eye-specific columns (uneven stripes) in the primary visual cortex. Thus, visual experience (i.e., neural activity) stimulates the growth and stabilization of neural connections, while lack of activity in one eye reduces the extent of neural connectivity from that eye to the visual cortex.

It has been proposed that ocular dominance plasticity is subject to both positive and negative regulatory controls. Positive effectors of ocular dominance plasticity include calmodulin-dependent kinase II and brain-derived neurotrophic factor. Recent studies showed that major histocompatibility complex class I (MHC1) is widely expressed in the brain, and that its expression is activity-dependent, despite the fact that it was previously thought to have little or no neuronal function. In contrast, MHC1 expression colocalizes with synaptic markers—in hippocampal neurons, including PSD-95 (Fig. 3), and it is expressed at a low but detectable level in cortical and thalamic neurons during development. Furthermore, expression of MHC1 is strongly downregulated by chronic activity blockade.

In order for MHC1 to form functional cell surface complexes, MHC1-expressing cells must also express β2-microglobulin (β2M) and the transporter associated with antigen processing (a TAP1/TAP2 heterodimer). Therefore, β2M–TAP1 double knockout (KO) mice show strongly reduced amounts of cell surface and intracellular MHC1 and these β2M–TAP1 knockout mice provide a useful system to study the function of MHC1 in activity-dependent neuronal plasticity. When the monocular and binocular zones of the visual cortex were measured in wildtype and β2M–TAP1 knockout mice with or without altered visual experience via one eye, the activity-dependent increase in the binocular zone, as measured by expression of Arc mRNA, was significantly greater in β2M–TAP1 knockout mice than in wildtype mice. This observation showed that β2M/TAP1 negatively regulates activity-dependent ocular dominance plasticity and indirectly implicated MHC1 molecules. To further explore a direct requirement for MHC1, KbDb mutant mice which lack just two of the more than 60 MHC1 genes were studied: ocular dominance plasticity was also increased in these mice, demonstrating that one or both of the MHC1 proteins acts to limit this form of synaptic plasticity.

Whole cell patch–clamp analysis of synapse functional parameters was also carried out in cultures of wildtype and β2M–TAP1 knockout hippocampal neurons. The results showed that mini excitatory postsynaptic current (mEPSC) frequency was 40% higher and the size of presynaptic boutons was modestly higher in β2M–TAP1 knockout neurons than in wildtype neurons, whereas postsynaptic parameters (PSD-95 puncta size and mEPSC amplitude) were normal in β2M–TAP1 knockout neurons. Interestingly, the phenotype of β2M–TAP1 knockout mice was very similar to the phenotype of mice with an inactivating mutation in PIRB (paired immunoglobulin-like receptor B), an MHC1 receptor expressed in cortical neurons. Together these observations suggest that the activity-dependent neuronal function of MHC1 may also require PIRB. These results support a model in which ocular dominance plasticity is positively and negatively regulated during development and in which MHC1 and PIRB are required for negative regulatory control of this process. The existence of molecules that function to limit the amount of activity-dependent plasticity in neuronal circuits is unexpected, and represents new opportunities for therapies following damage to the nervous system.

Eve Marder Waltham, MA, US

Beyond optimality: how good is good enough? The pyloric rhythm of the crustacean stomatogaster ganglion (STG) has been used as a test-bed functional neurological circuit. Advantages of this system include the fact that the STG is composed of only 30 neurons, that the connectivity diagram of the circuit is precisely known, and that it generates stereotyped motor patterns, including the pyloric rhythm. The characteristically triphasic pyloric rhythm is produced by the pacemaker anterior burster (AB) neuron, which is electrically coupled to two pyloric dilator (PD)

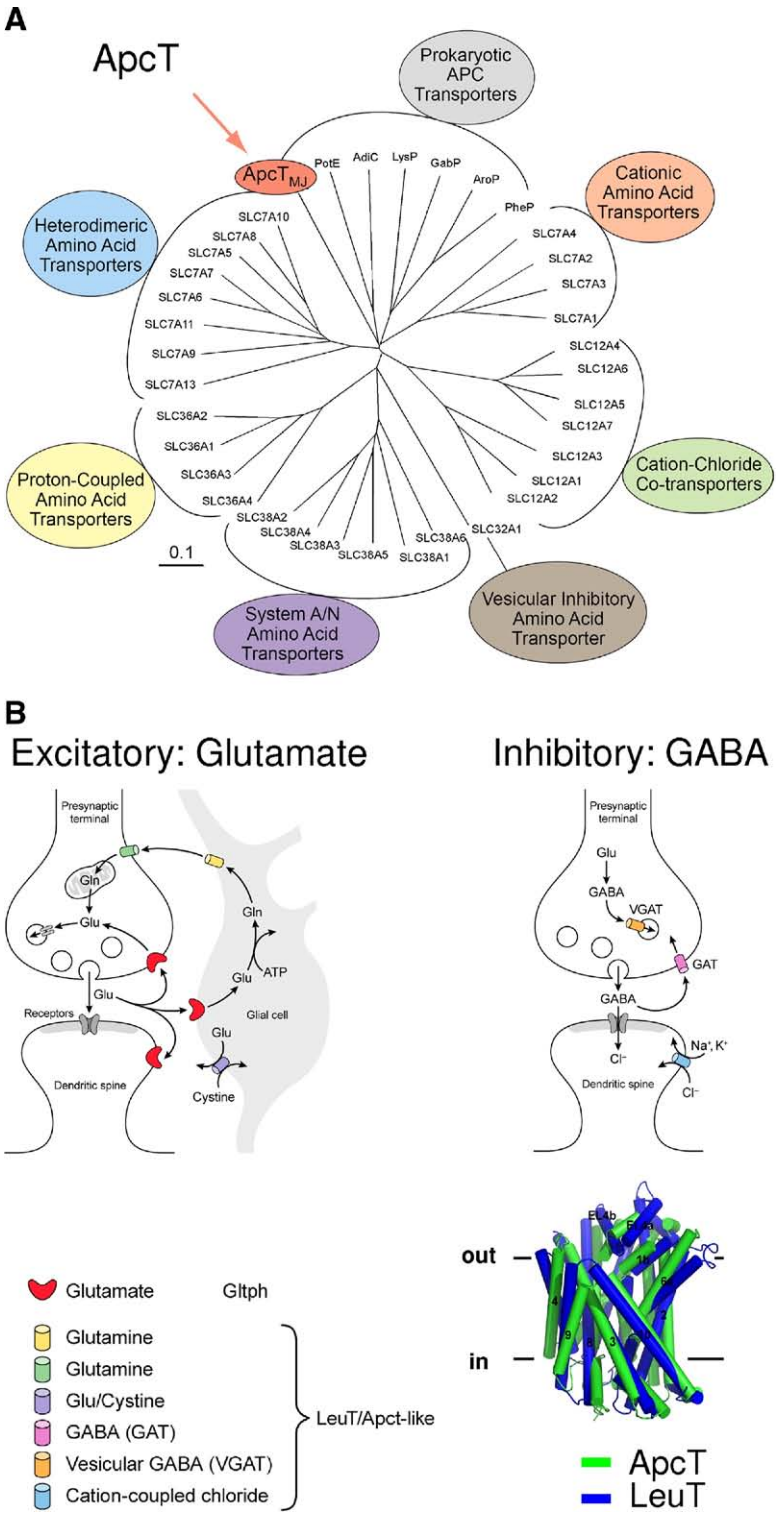

Fig. 2. The amino acid/polyamine/organocation (APC) transporter superfamily. A. ApcT is a bacterial ortholog of the glutamate/cystine antiporter. The figure presents a phylogenetic tree for the APC superfamily, including representative members of seven families. Each family stems from a point near the center of the unrooted tree. B. This superfamily comprises important transporters (light colors) at excitatory and inhibitory synapses, whereas the glutamate transporters at the plasmalemma (red) and vesicular membranes (grey) belong to different families. Chrystal structure determination shows that two representative members (LeuT and ApcT) have the same architecture. (Provided by Eric Gouaux.)

neurons, a single lateral pyloric (LP) neuron, and five to eight pyloric (PY) neurons. For many simulations and analyses of the pyloric rhythm, the AB and PD neurons are considered as a single bursting unit (AB/PD). The pyloric

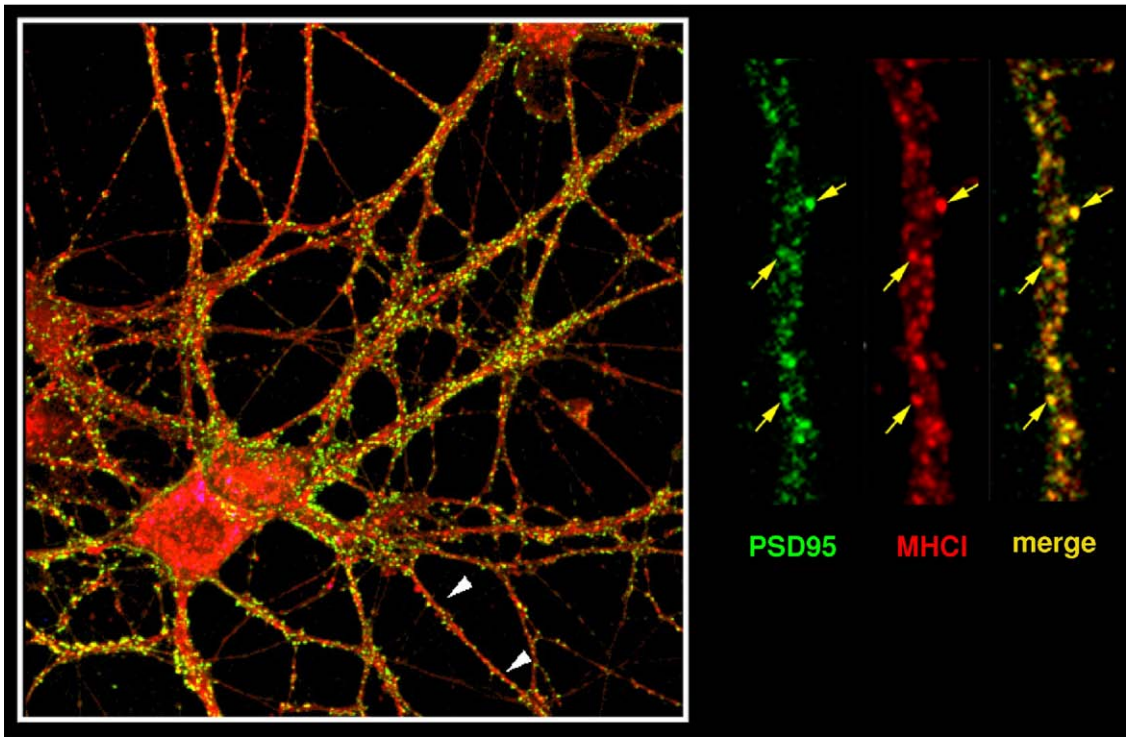

Fig. 3. MHC1 colocalizes with PSD-95 in hippocampal neurons. Anti-MHC1 antibodies were detected with Cy3 (red), and anti-PSD-95 with Cy5 (green). Left panel shows MHC1 immunoreactivity in hippocampal dendrites and spines. Right panels show PSD-95 immunostained puncta marked by arrows. Merged fields show colocalization of PSD-95 and MHC1 signals. (Provided by Carla J. Shatz, and adapted with permission from Goddard et al., 2007 *Proc Natl Acad Sci USA*.)

network continuously generates triphasic activity bursts in the order LP–PY–PD, and the STG as a unit continues to cycle rhythmically for several days when maintained under appropriate *in vitro* conditions. Interestingly, STGs from juvenile and adult crustaceans differ in physical size many-fold without significant impact on the consistency of the pyloric rhythm. This suggests that circuit performance may have significant tolerance for variation in geometry and/or synaptic parameters, in the context of this model neurological circuit.

It has been proposed that neuronal circuits tolerate significant biological variation, and that circuit performance remains high despite significant variation in circuit parameters. This hypothesis has been tested both computationally and experimentally using the STG and the pyloric rhythm as a model system. Computational simulations were made for >20 million model pyloric circuits, in which the strength of each synapse was varied independently from 0 to 100 nS (0, 3, 10, 30 or 100 nS), and the intrinsic properties of neurons in the model circuit were varied independently. Neuronal properties used in these simulations were based on a database of empiric measurements in STG circuits. As a reference point for evaluating the behavior of the simulated circuits, the pyloric rhythm was recorded and analyzed in 99 lobsters, and the experimentally-observed value range was determined for 15 critical circuit parameters (i.e., cycle period, burst duration, firing gap, firing delay, etc.). Using these parameter value ranges as criteria, approximately 20% of the 20 million computationally-defined model circuits were "pyloric-like", and 11% of these models (452,000) were "pyloric" (i.e., predicted values were within the range of experimentally-observed values). These data indicate that circuit performance tolerates significant diversity in synaptic strength and intrinsic neuronal properties (Fig. 4). For some circuit parameters, several orders of magnitude variation can be tolerated. It is possible that this level of variation provides a substrate for natural selection, and thus confers evolutionary advantage in the context of diverse environmental conditions.

The expression of four potassium channels (Shal, BK–KCa, Shab, Shaw) a hyperpolarization-activated inward current channel (IH) and a sodium channel (Para) was also analyzed by quantitative RT–PCR in six neuronal cell types (GM, IC, LP, LG, LPG, and PD). The level of expression of each channel in the same cell type varied 3 to 5-fold from animal to animal (interanimal variability). However, the relative expression of different channels varied according to cell type and was highly correlated, such that a unique pattern of channel gene expression was associated with each cell type. This result suggests that one might be able to infer neuronal cell identity by quantifying expression of a small number of cell-type specific channel genes.

Fred Gage San Diego, CA, USA

Regulation and function of neurogenesis in the adult mammalian brain. Early models of brain structure and function suggested that connectivity in the adult brain

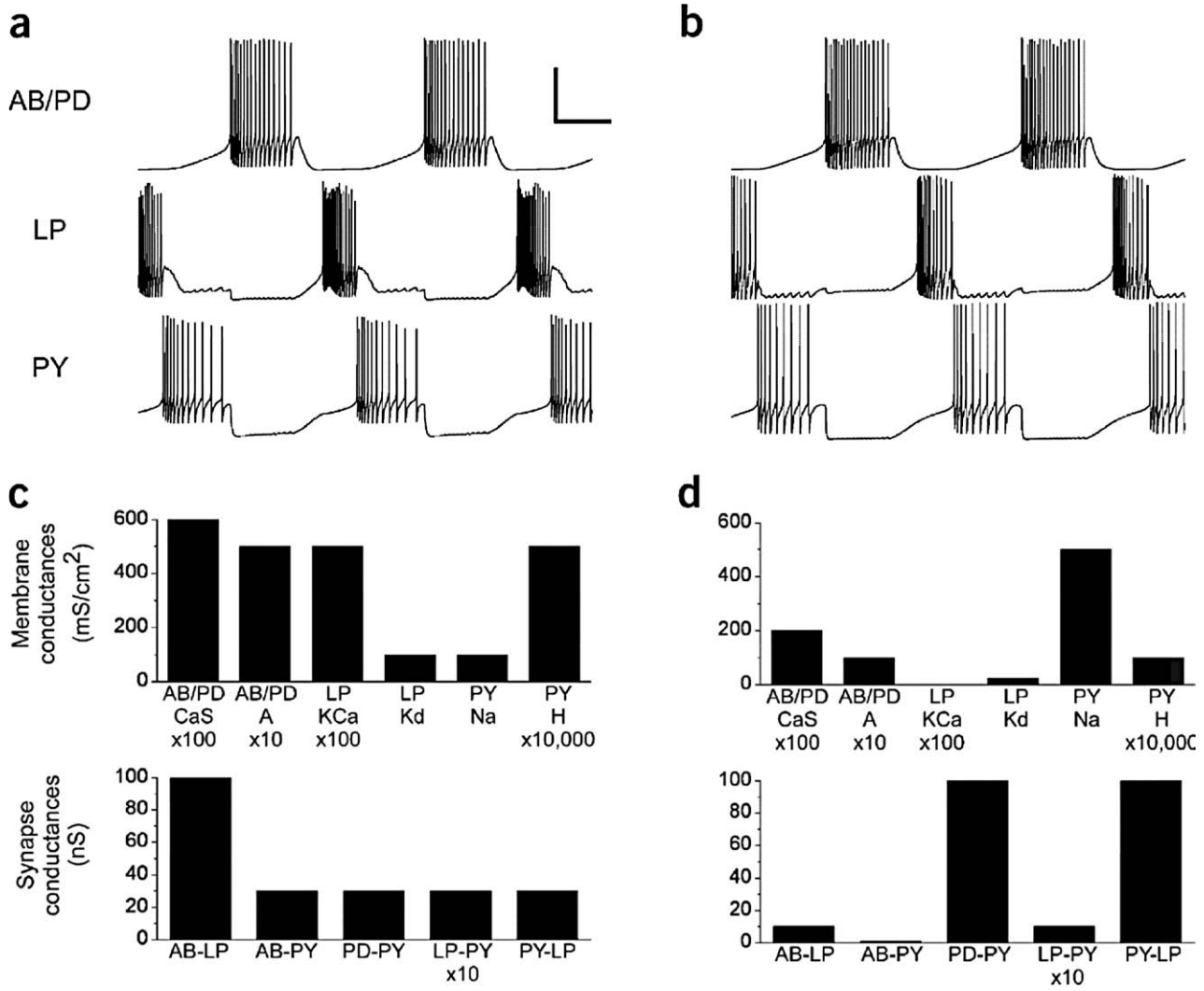

Fig. 4. Similar model-network activity from different network properties. (a, b) Voltage traces from two model pyloric networks. The two networks generated very similar activity in spite of widely differing cellular and synaptic properties. (c, d) Selected membrane conductances (top) and synaptic conductances of the model network shown in a and b, respectively. The model neurons shown here (AB/PD, LP, PY, see text) feature a Na^+ current (Na); a slow transient Ca^{2+} current (CaS); a transient K^+ current (a); a Ca^{2+}-dependent K^+ current (KCa); a delayed rectifier k^+ current (KD); and a hyperpolarization-activated inward current (H). Scale bars=0.5 s and 50 mv. (Reproduced with permission from Prinz et al., 2004 *Nat Neurosci*.)

was static or diminished over time, but that it did not increase. In other words, it was thought that the capacity to form new synapses was lost by the time the brain reached its mature adult form. In contrast, recent studies indicate that the adult brain has a significant degree of plasticity and that neurons are continuously born from neural stem cells in the subgranular zone of the hippocampal dentate gyrus and in the subventricular zone of the lateral ventricles.

A novel high resolution retrovirus-mediated method has been used to follow neurogenesis in the adult mouse brain, and to map the connectivity and fate of nascent neurons in the dentate gyrus. This method exploits the fact that green fluorescent protein (GFP)-expressing replication-deficient Moloney murine leukemia virus can be directly injected into the mouse dentate gyrus, where it only integrates (and generates GFP) in actively dividing cells. Using this method, adult hippocampal neurogenesis has been detected (as GFP fluorescence) in mouse (Fig. 5), confirming and extending evidence for neurogenesis in rat, cat, birds, tree shrew, marmoset, rhesus monkeys and humans. Interestingly, adult neurogenesis is regulated by external stimuli; more nascent neural cells are generated and make synapses in response to mental stimulation or physical exercise (such as running on a treadmill) and fewer in response to stress and aging.

Immunoelectron microscopic studies in the dentate gyrus showed that GFP-labeled nascent neurons form axo-dendritic and axo-spinous synapses with hilar interneurons and CA3 neurons by the third week of neurogenesis. Nascent neuronal spines appear to target pre-existing synapses, and computational studies suggest that this phenomenon is non-random. To test the function of these nascent synaptic connections, light-gated cation channel

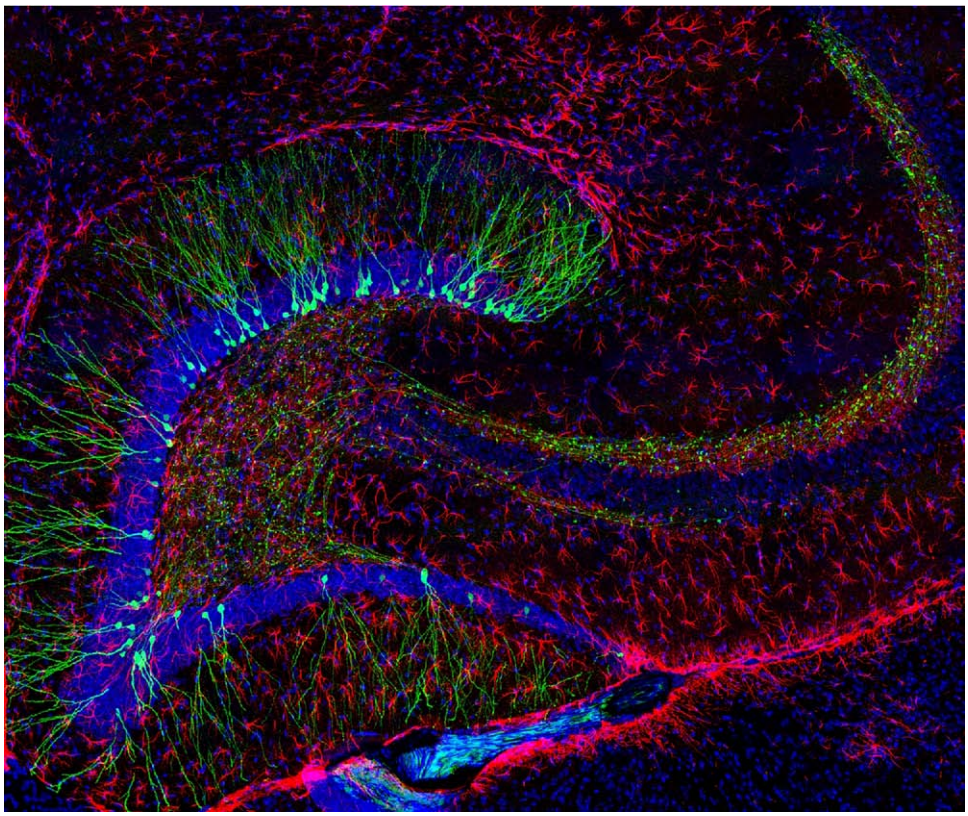

Fig. 5. Adult neurogenesis in the mouse hippocampus. The retroviral vector expressing GFP from the CAG promoter (a combination of chicken beta-actin promoter and cytomegalovirus immediate-early enhancer) was injected into the dentate gyrus of 6 to 7-week-old mice. Immunofluorescence confocal microscopy was used to visualize GFP-labeled (green) adult-born neurons in hippocampal sections 17 days post-injection. Astrocytes (red) and nuclei (blue) are shown. (Provided by Fred H. Gage with thanks to Chunmei Zhao and Kim Strecker.)

channelrhodopsin-2 (ChR2) was expressed in the progeny of dividing neural precursors in the adult dentate gyrus, and the post-synaptic response to light stimulation was quantified in acute hippocampal slices. The results demonstrated that the newly formed synapses are fully functional, as measured by release of glutamate onto postsynaptic targets in the dentate gyrus.

Adult neurogenesis has also been examined in transgenic mice expressing GFP from the neuro-specific promoter for transcription factor Sox2, a marker of embryonic stem cells and neural stem cells. Initial studies revealed that GFP is expressed in cells with both radial and non-radial morphology in the granular zone of the dentate gyrus, and that GFP and Sox2 expression persists in a subfraction of non-radial cells. These data suggest that Sox-2 expressing neural cells are capable of self-renewal, in this model system. To examine stem cell fate and monitor neural stem cell differentiation over time, transgenic mice with a Flox-stop-inactivated GFP cassette were injected with virus expressing a Sox2-promoter driven GFP–Cre-recombinase fusion protein. This allowed regulated activation of GFP-expressing neural cell progenitors. Cell-type specific markers (i.e., BLBP, PROX1, DCX, GFAP, S-100b) and incorporation of BrdU were then monitored at multiple time points after Cre recombinase-mediated activation of GFP. The results indicated that Sox2-expressing cells in the dentate gyrus have the capacity for self-renewal as well as the capacity to give rise to neurons and astrocytes. Although these neural stem cells did not spontaneously differentiate into oligodendrocytes in this model system, enforced expression of achaete–scute complex homolog-like 1 (Ascl1) induced them to express oligodendrocyte markers (i.e., myelin basic protein, NG2, glutathione-S-transferase–π, oligodendrocyte transcription factor 2) in a context-dependent manner. Interestingly, forced expression of Ascl1 had no effect on neural stem cells *in vitro* or in the subventricular zone. This suggests that neural stem cell fate is not immutable, but is re-programmable, in a context-dependent manner.

Tim Bliss London, UK

The future of the past: new approaches to exploring the role of the hippocampus in memory. Long-term potentiation of chemical synaptic transmission was first demonstrated in anaesthetized rabbits, when it was observed that high frequency trains of electrical stimulation (tetani) to the hippocampus resulted in a sustained increase in the amplitude of field EPSPs at perforant path synapses in the dentate gyrus. In subsequent studies, the mechanisms of LTP have been examined in hippocampal slices maintained *in vitro*. In this system, tetani are usually delivered to Schaffer collateral/commissural fibres projecting from CA3 to CA1 pyramidal neurons. In more recent experiments, LTP has been detected at individual synapses in cultured

hippocampal slices using confocal microscopy and an intracellular fluorescent calcium indicator. This system has revealed that changes in release probability underlie LTP at single non-silent synapses and has provided evidence that single hippocampal synapses can exhibit multiple stable levels of potentiation or depression, and that LTP induced at silent synapses is expressed by postsynaptic mechanisms.

LTP is thought to be involved in memory, at least in part because it is a mechanism by which activity can rapidly induce long term change in brain function. RG Morris and his colleagues have formalized this idea in a synaptic plasticity and memory (SPM) hypothesis, which states that "activity-dependent plasticity is induced at hippocampal synapses during memory formation, and is both necessary and sufficient for the information storage underlying hippocampus-dependent memory." If the SPM hypothesis is correct, it should be possible to demonstrate the following: (1) agents that block LTP block learning; (2) learning is associated with the induction of LTP; (3) reversal of LTP reverses memory and causes forgetting; and (4) selective potentiation of a neural circuit induces memory. While the SPM hypothesis is now widely accepted, strong empiric evidence in support of the SPM hypothesis and its predictions has been difficult to obtain by reductionist methods commonly used to study LTP, learning and memory. Therefore, new approaches are needed to test the SPM hypothesis.

Molecular genetic approaches may provide tools for selectively inhibiting or modulating synaptic activity in memory-specific neural subnetworks, and for testing the SPM hypothesis and its predictions. This approach distinguishes itself from earlier approaches, in that it proposes to manipulate a group of synapses (a neural assembly) specifically involved in encoding a hippocampus-dependent memory. Specific "immediate early genes", including Zif268 and Arc, are upregulated in hippocampal neurons within 30 min after the induction of LTP. The promoters of immediate early genes, which have been cloned and characterized, could be used to drive LTP-dependent expression of agents that block, potentiate or label neurons involved in an LTP-associated memory. Using this approach, it may be possible to identify hippocampal neurons that encode a specific memory, to destroy a specific memory, or to induce LTP/memory in a neural subnetwork. In order to selectively label hippocampal neurons involved in a specific memory, one could induce LTP in transgenic mice that express ferritin, an MRI-contrast enhancing agent, from the Zif268 promoter, and then track memory induction using high resolution MRI. Similarly, in order to selectively erase a memory, one could induce LTP in transgenic mice that express an ivermectin-sensitive chloride channel from the Zif268 promoter, and subsequently silence the network of neurons activated by and encoding that memory by treating the mice with ivermectin. These novel and powerful approaches are currently being developed to test, and hopefully prove or disprove, at a network level the predictions of the SPM hypothesis.

Peter Seeburg Heidelberg, Germany

Dissection of hippocampal information processing by genetic AMPA and NMDA receptor subtype manipulation in the mouse. The hippocampus is thought to play a significant role in learning and memory through acquisition, storage and recall of spatial and context-dependent information. Hippocampus-dependent learning requires glutamatergic and GABA-ergic receptors, which are involved in mediating activity-dependent synaptic plasticity. Three glutamate receptor subtypes have been identified in the brain: the AMPA receptor, the *N*-methyl-D-aspartate (NMDA) receptor, and the kainate receptor. These receptor subtypes are expressed in distinct brain regions and are differentially sensitive to several glutamate antagonists and analogues. AMPA and NMDA receptors are multimeric proteins with multiple isoforms, and the AMPA and NMDA receptor subunits are encoded by the *GluR-A*/*GluR-B*/*GluR-C*/*GluR-D* (also known as *GluR1*/*GluR2*/*GluR3*/*GluR4*) and *NR1*/*NR2A*/*NR2B* genes, respectively.

The role of AMPA and NMDA receptors in learning and memory has been examined in mutant mice with constitutive or conditional knockout of one or more receptor subunits in distinct brain regions. The functional effects of partial or complete receptor knockout have been compared by measuring performance of wildtype and mutant mice in a series of information processing tasks. These tasks include the Morris water maze, Y-maze and radial mazes, which test spatial reference memory, the T-maze, which tests spatial working memory, as well as tasks that test pattern separation, pattern completion, contextual fear conditioning and cued fear conditioning. All of these information processing tasks require glutamatergic synaptic transmission in the hippocampus, as indicated by the fact that mice with hippocampal lesions are unable to perform any of these learning tasks, while sham-operated mice perform as well as wildtype mice.

GluR-A is expressed throughout the mouse brain, but most prominently in the hippocampus, where the GluR-A/GluR-B heteromer is the most abundant AMPA receptor isomer. *GluR-A* knockout mice develop normally and are viable as adult animals; however, *GluR-A* knockout mice display reduced synaptic strength and altered distribution of GluR-B in the hippocampus. Tetanus-induced field LTP is absent in *GluR-A* knockout mice, but spike-time-dependent LTP appears to be normal. Performance on tasks that require spatial reference memory (Morris water maze, Y-maze and six-arm radial maze) is normal, while performance on tasks that require spatial working memory (T-maze) is severely impaired. Although hippocampal lesions ablate both types of learning, GluR-A appears to be required only for the latter, which is dependent on short-term habituation instead of incremental associative learning. Interestingly, *GluR-A* knockout mice show improved long-term habituation relative to wildtype mice, suggesting that short-term habituation could interfere with associative long-term habituation in certain contexts.

NMDA receptors are glutamate-gated cation channels with high Ca^{2+} permeability that are essential for synaptic

plasticity in glutamatergic neurons in the forebrain. The primary NMDA receptor subtypes in these neurons of adult mice are NR1/NR2A and NR1/NR2B receptors. NR2B is also expressed during embryonic development and is essential for viability. NR2A is only expressed postnatally, and it is not required for viability. Because mice survive with global knockout of NR2A, as shown by Prof. Mishina's laboratory, or upon conditional Cre recombinase-mediated forebrain-specific knockout of *NR2B*, these mutant animals can be used to analyze the role of NMDA receptors in learning and memory.

The phenotype of mice with global knockout of *NR2A* is similar to, but somewhat less severe than, the phenotype of *GluR-A* knockout mice. In particular, *NR2A* knockout mice demonstrate nearly normal tetanus-induced field LTP, impaired pairing-induced cellular LTP, robust spatial reference memory (Morris water maze, Y-maze, radial maze), and selective loss of spatial working memory (T-maze). In contrast, forebrain-specific knockout of *NR2B* results in severe impairment in both spatial reference memory (Morris water maze, Y-maze, radial maze) and spatial working memory (T-maze), as well as impaired performance on non-spatial cognitive tests, including novel object recognition and visual discrimination learning, which are conserved in hippocampal-lesioned animals. However, when *NR2B* knockout is restricted to principal cells of dorsal CA1 and dentate gyrus granule cells, a much less severe phenotype is observed, which includes selective deficits in short-term habituation and spatial working memory, but intact spatial reference memory.

Susumu Tonegawa Cambridge, MA, USA

Hippocampal circuits and episodic learning and memory. The hippocampus plays a role in multiple types of episodic memory, including spatial memory and contextual fear memory. Region specific knockout of NMDA receptor subunit, NR1, in the hippocampus has implicated the CA3 region in pattern completion and rapid encoding, and the dentate gyrus in pattern separation.

The hippocampus has two parallel excitatory pathways: the trisynaptic pathway consists of a loop from the entorhinal cortex via the dentate gyrus → CA3 → CA1 and

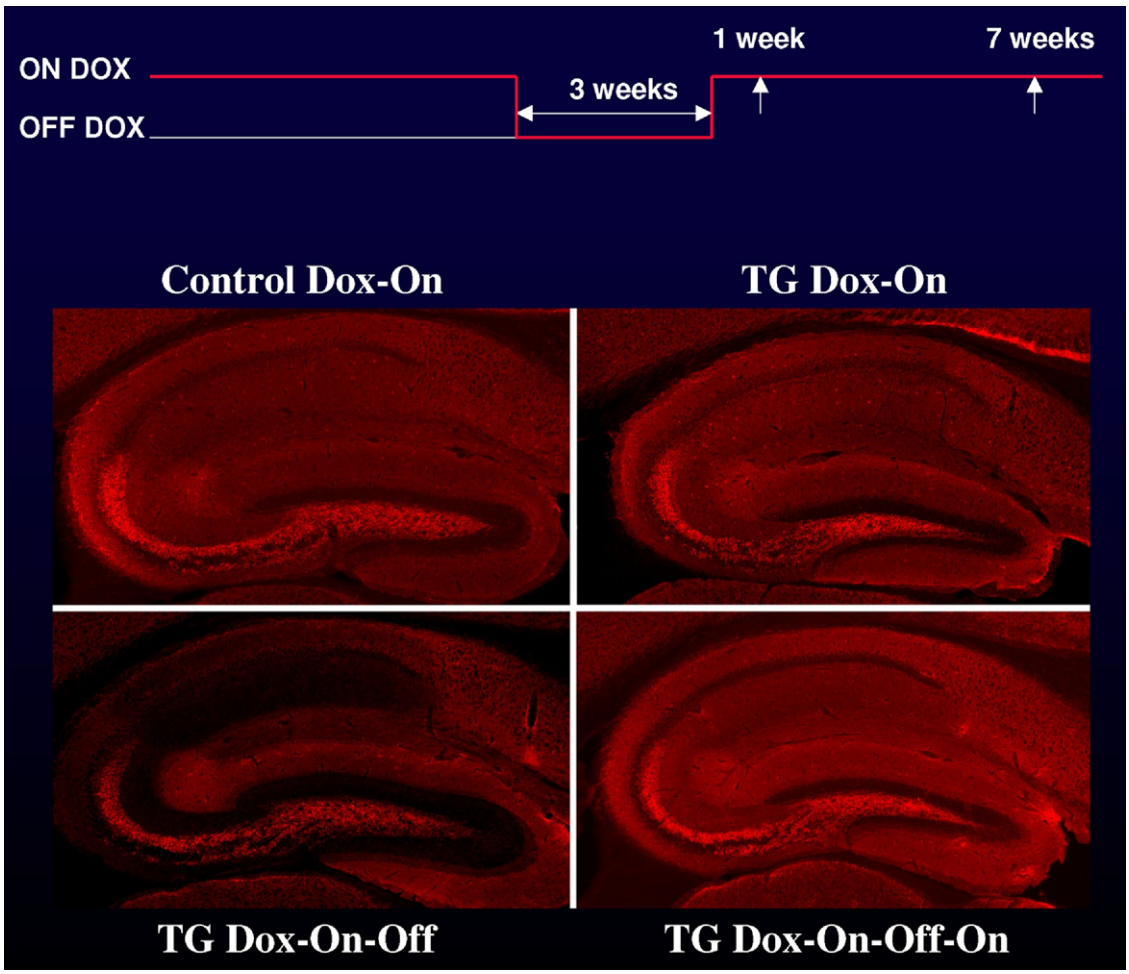

Fig. 6. Selective TeTX-mediated doxycycline-regulated degradation of VAMP2 in CA3 pyramidal neurons. Immunofluorescence staining with VAMP2 antibodies was performed on hippocampal sections from CA3–TeTX transgenic or control. Mice were either maintained on a doxycycline (Dox)-containing diet, or cycled through Dox-on Dox-off periods, as indicated in the experimental time-line at the top of the figure. (Adapted with permission from Nakashiba et al., 2008 *Science*.)

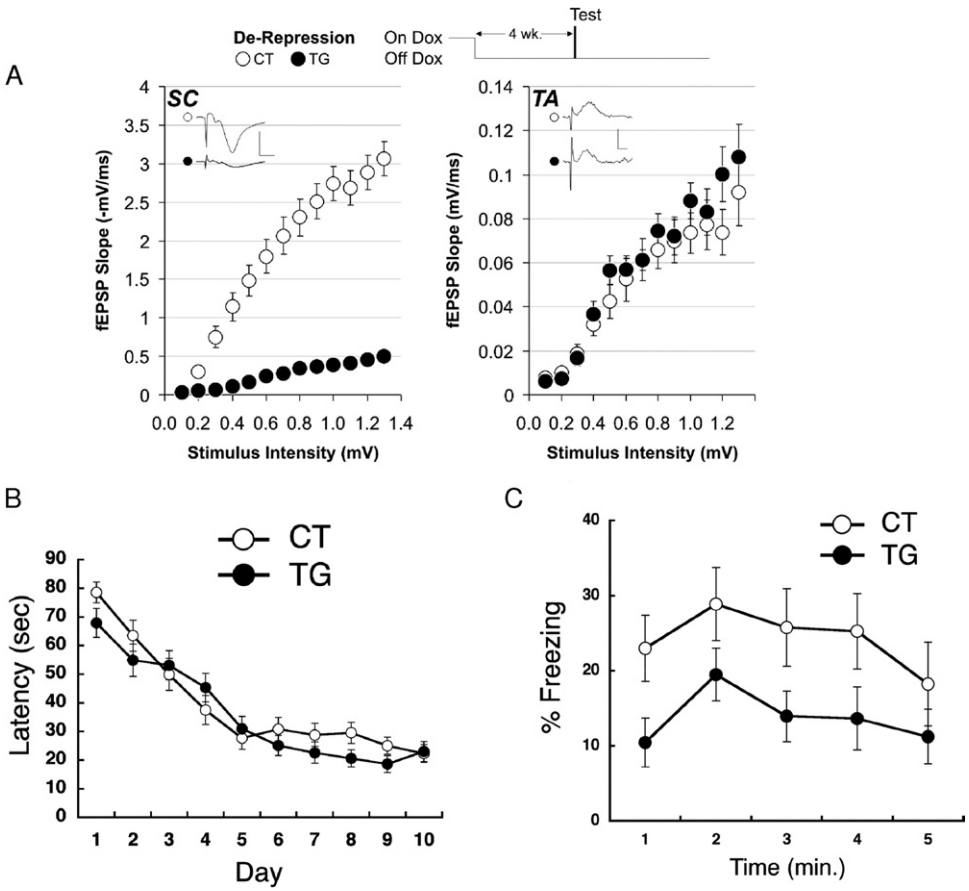

Fig. 7. Characterization of trisynaptic-loop deficient transgenic mice. A. Input–output relationships of SC and TA inputs to CA1 in CA3–TeTX (TG) mice and control (CT) littermates after 4 weeks of doxycycline (Dox) withdrawal. Sample traces are representative of recorded mean maximal fEPSP slopes. B. Performance in Morris Water Maze test for TG mice and control littermates after 4 weeks of Dox withdrawal. C. Performance in contextual fear conditioning (kinetics of averaged freezing and re-freezing over 5 min) for TG mice and control littermates after 4 weeks of Dox withdrawal. (Adapted with permission from Nakashiba et al., 2008 *Science*.)

back to the entorhinal cortex, while the monosynaptic pathway carries information in a loop from the entorhinal cortex via CA1 back to the entorhinal cortex. The differential role of the tri- and mono-synaptic pathways in different memory subtypes and aspects was determined by selectively inactivating synaptic transmission in a spatially-restricted, cell type-specific, temporally-regulated manner. For instance, this was achieved by selective expression of a transgenic tetanus toxin gene that is restricted to CA3 pyramidal cells in a temporally controllable manner. These mice are normal when maintained on a diet containing doxycycline. However, in the absence of doxycycline, VAMP2 is selectively degraded in CA3 pyramidal cells, and trisynaptic pathway-dependent brain function is blocked, while monosynaptic pathway-dependent brain functions are unaffected (Fig. 6). These animals perform normally in incremental spatial learning tasks, such as the Morris water maze, but are impaired in rapid one-trial learning of a novel context (contextual fear conditioning) and pattern completion-based memory recall (Fig. 7). These behavioral phenotypes correlate well with the results of in vivo recordings of CA1 pyramidal cells: in a novel environment the tuning of mutants' CA1 place cells is severely impaired while the repeated exposure to the environment significantly improves the spatial tuning. These data demonstrate that the trisynaptic pathway is required for rapid contextual learning, while the monosynaptic pathway is sufficient for slow, associative multi-trial learning tasks.

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